

# 13

## Thin layer chromatography

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### KEYPOINTS

#### Principles

- An analyte migrates up or across a layer of stationary phase (most commonly silica gel), under the influence of a mobile phase (usually a mixture of organic solvents), which moves through the stationary phase by capillary action. The distance moved by the analyte is determined by its relative affinity for the stationary vs the mobile phase.

#### Applications

- Used to determine impurities in pharmaceutical raw materials and formulated products
- Often used as a basic identity check on pharmaceutical raw materials.
- Potentially useful in cleaning validation, which is part of the manufacture of pharmaceuticals.

#### Strengths

- Detection by chemical reaction with a visualisation reagent can be carried out, which means that more or less every type of compound can be detected if a suitable detection reagent is used.
- Robust and cheap.
- In conjunction with densitometric detection, it can be used as a quantitative technique for compounds which are difficult to analyse by other chromatographic methods because of the absence of a chromophore.
- Since all the components in the chromatographic system can be seen, there is no risk, as is the case in gas chromatography (GC) and HPLC analyses, that some components are not observed because they do not elute from the chromatographic system.
- Batch chromatography can be used to analyse many samples at once, increasing the speed of analysis, and can be automated.

- The method is flexible since thin layer chromatography (TLC) plates can be simply treated with a variety of chemicals thus imparting a wide range of properties to the stationary phase.

#### Limitations

- The number of theoretical plates available for separation is limited in routine TLC systems, although high performance TLC (HPTLC) plates can offer nearly the same efficiency in a 10 cm distance as an HPLC column of the same length
- Sensitivity is often limited
- Not suitable for volatile compounds
- Requires more operator skill for optimal use than HPLC.

## Introduction

Thin layer chromatography (TLC) has developed into a very sophisticated technique for identification of compounds and for determination of the presence of trace impurities. Since it was one of the earliest chromatographic techniques, a huge array of TLC-based tests is available and pharmacopoeial monographs reflect the extent to which this technique has been developed as a fundamental quality control technique for trace impurities. The reason for its prominence in this regard is due to its flexibility in being able to detect almost any compound, even some inorganic compounds. Following TLC, the entire chromatogram can be seen and thus there is no doubt over whether or not components in a sample have failed to elute from a chromatographic system as is the case with HPLC and GC, and even capillary electrophoresis (CE). In this short chapter it would be impossible to outline all of the tests that can be used; comprehensive reviews of the technique have been written.<sup>1,2</sup> Even the most advanced form of TLC, high performance TLC (HPTLC), remains essentially a simple technique. The sophistication in the application of the technique derives from the broad choice of stationary phases, mobile phases and the wide range spray reagents which can be used for visualising the chromatogram.

## Instrumentation

Figure 13.1 shows a simple thin layer chromatography apparatus.

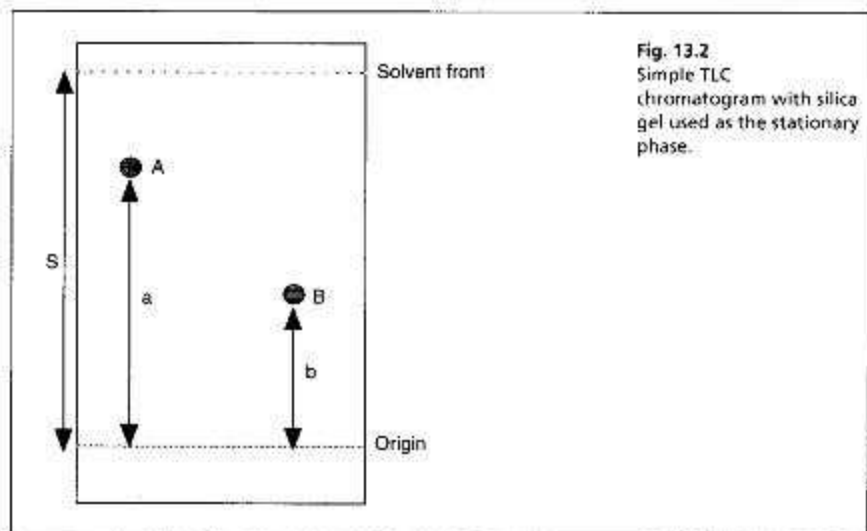
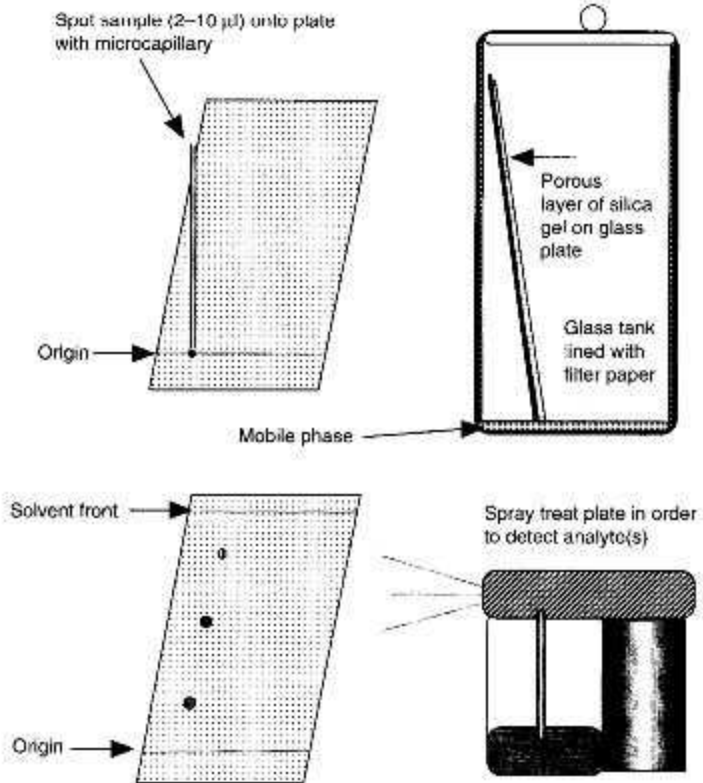
The most frequently used system is a glass or plastic plate coated with silica gel; for routine applications the silica gel particle size is in the range 2–25  $\mu\text{m}$ . The method of use for this system is as follows:

- A few  $\mu\text{l}$  of sample solution are slowly spotted onto the plate at the origin. If more than *ca* 1  $\mu\text{l}$  is applied at once, the spot will spread too far. The spot has to be allowed to dry between each application of 1  $\mu\text{l}$ . Loadings of sample are typically 20  $\mu\text{g}$ .
- The bottom 0.5 cm of the plate is immersed in the mobile phase contained in a tank and the liquid mobile phase is allowed to travel up the silica gel plate by capillary action.
- The more polar a compound is the more it adsorbs (partitions into) the silica gel stationary phase, the less time it spends in the mobile phase as it travels up the plate and thus the shorter the distance it travels up the plate in a given time.

## TLC chromatogram

A diagram of a typical thin-layer chromatography plate after development and spraying to locate the analytes is shown in Figure 13.2.

**Fig. 13.1**  
Basic apparatus for thin-layer chromatography.



**Fig. 13.2**  
Simple TLC  
chromatogram with silica  
gel used as the stationary  
phase.

In Figure 13.2, compound A is less polar than compound B since it travels further with the mobile phase in the same time. The distance travelled by the compound from the origin (where the compound is put onto the plate) divided by the distance travelled by the solvent from the origin is called the 'R<sub>f</sub> value' of the compound. For example, for compound A,  $R_f = a/S$ ; for compound B,  $R_f = b/S$ ; the R<sub>f</sub> is usually quoted as a  $R_f \times 100$  value. The area/intensity of a spot on a TLC plate is logarithmically related to the concentration of the analyte producing it.

### Self-test 13.1

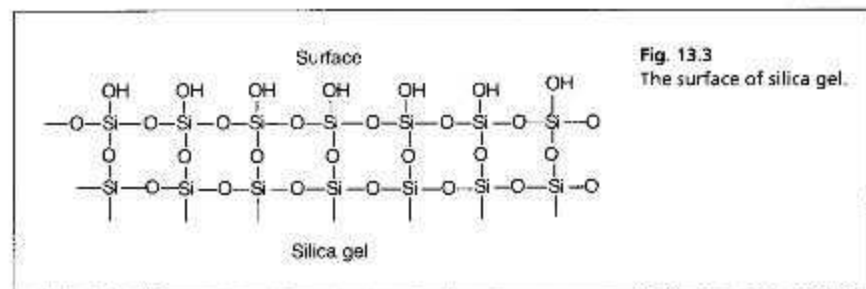
Solvent is allowed to move 10 cm from the origin up a TLC plate. The time taken to develop the plate for this distance is 15 min. On the basis of this information, complete the table below.

Distance moved by analyte	R <sub>f</sub> × 100 value	Time spent in mobile phase	Time spent in stationary phase
(i) 8 cm			
(ii) 6 cm			
(iii) 4 cm			

Answers: (i) 80, 12, 3; (ii) 60, 9; (iii) 40, 6, 9

## Stationary phases

Silica gel (Fig. 13.3) is the most commonly used adsorbant for TLC. The rate at which compounds migrate up a silica gel plate depends on their polarity. In a given length of time, the most polar compounds move the least distance up the plate while the least polar move the furthest.



Although silica gel is used widely, some other polar stationary phases are also used in pharmacopoeial tests; silica gel may also be used in modified form. Some examples of stationary phases are given in Table 13.1.

## Elutropic series and mobile phases

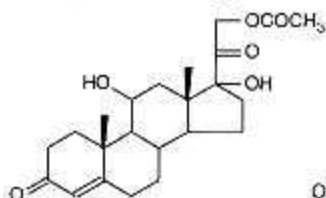
As described in Chapter 12, the strength of a mobile phase depends on the particular solvent mixture used. Table 13.2 lists common solvents in order of increasing polarity. The more polar a solvent or solvent mixture, the further it will move a polar compound up a silica gel TLC plate. When non-polar compounds are being analysed, there will not be a marked increase in the distance migrated with increasing polarity of the mobile phase since they migrate towards the solvent front under most conditions. Although water is polar, there are practical difficulties in using pure water as a solvent since many organic compounds are not very soluble in water; thus

Table 13.1 Stationary phases which are commonly used in TLC

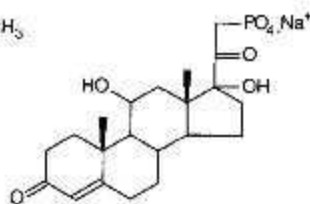
Stationary phase	Description	Applications
Silica gel G	Silica gel with average particle size $15\ \mu\text{m}$ containing ca 13% calcium sulphate binding agent	Use in a wide range of pharmacopoeial tests. In practice commercial plates may be used which contain a different type of binder
Silica gel GF <sub>254</sub>	Silica gel G with fluorescent agent added	The same types of applications as silica G where visualisation is to be carried out under UV light
Cellulose	Cellulose powder of less than $30\ \mu\text{m}$ particle size	Identification of tetracyclines
Keiselguhr G	Diatomaceous earth containing calcium sulphate binder	Used as a solid support for stationary phases such as liquid paraffin used in analysis of fixed oils

## Self-test 13.2

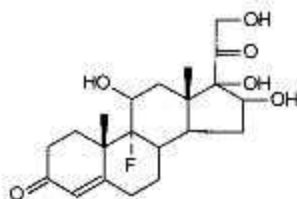
The steroids below are spotted onto a silica gel TLC plate. The plate is developed in methylene chloride/ether/methanol/water (77:15: 8:1:2) and under UV light has the appearance shown in Figure 13.4. From your knowledge of the polarity of organic molecules, match the steroids to the spots on the TLC plate shown in Figure 13.4.



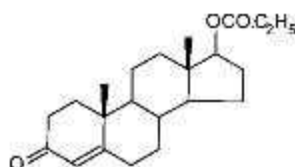
Hydrocortisone acetate



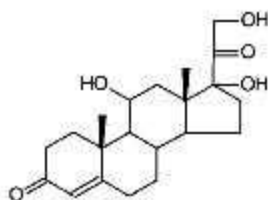
Hydrocortisone sodium phosphate



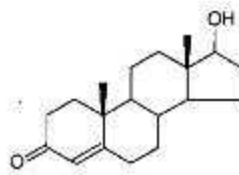
Triamcinolone



Testosterone propionate



Hydrocortisone



Testosterone

Answer: 1. hydrocortisone sodium phosphate; 2. triamcinolone; 3. hydrocortisone; 4. hydrocortisone acetate; 5. testosterone; 6. testosterone propionate

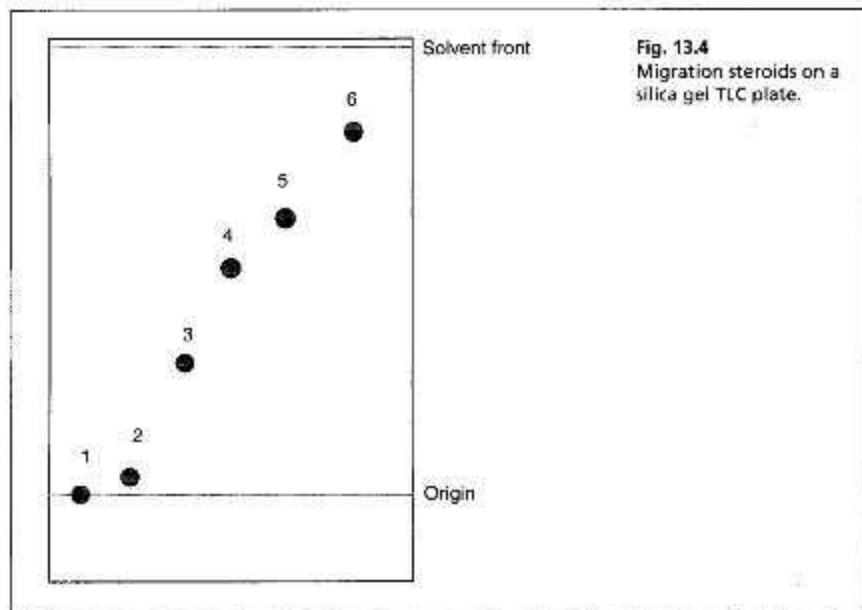


Table 13.2 Elutropic series

Solvent	Polarity index
Hexane (C <sub>6</sub> H <sub>14</sub> )	0
Toluene (C <sub>7</sub> H <sub>8</sub> )	2.4
Diethylether (C <sub>4</sub> H <sub>10</sub> O)	2.8
Dichloromethane (CH <sub>2</sub> Cl <sub>2</sub> )	3.1
Butanol (C <sub>4</sub> H <sub>9</sub> OH)	3.9
Chloroform (CHCl <sub>3</sub> )	4.1
Ethyl acetate (C <sub>4</sub> H <sub>8</sub> COOCH <sub>3</sub> )	4.4
Acetone (CH <sub>3</sub> COCH <sub>3</sub> )	5.1
Methanol (CH <sub>3</sub> OH)	5.1
Ethanol (C <sub>2</sub> H <sub>5</sub> OH)	5.2
Acetonitrile (CH <sub>3</sub> CN)	5.8
Acetic acid (CH <sub>3</sub> COOH)	6.2
Water (H <sub>2</sub> O)	9.0

it is usually used in mobile phases containing a water miscible organic solvent such as methanol. Quite subtle changes in separation can be achieved by using complex mixtures of solvents. Because of its simplicity, TLC is often used as a preliminary screen for identifying drugs and thus mobile phases have been developed which ensure that a particular drug will have a quite different R<sub>f</sub> value in one system compared with another.

For example, in a general screen for acidic drugs, which includes most of the NSAIDs (Fig. 13.5), three mobile phases may be used.<sup>1</sup> Table 13.3 shows the R<sub>f</sub> values obtained for three NSAIDs in three different mobile phases. It can be seen from the data in Table 13.3 that even for closely related structures slight differences in polarity and lipophilicity can be exploited to produce separation. For instance, ibuprofen is the least polar drug in system 1 but is the most polar drug in system 3, where the carboxyl groups in the structures will be ionised due to the ammonia in the mobile phase. It can also be seen that the polarity of a mobile phase containing a mixture of chloroform and acetone is similar to that of pure ethyl acetate.

Fig. 13.5  
Some NSAIDs.

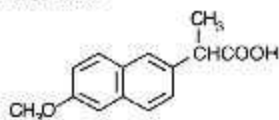


Table 13.3

Mobile phase	diclofenac Rf	mefenamic acid Rf	ibuprofen Rf
1. Chloroform/acetone (4:1)	25	41	46
2. Ethyl acetate	40	54	54
3. Ethyl acetate/methanol/strong ammonia solution (80:10:10)	29	32	18

### Self-test 13.3

Considering the three solvent systems (1, 2 and 3) given in Table 13.3, indicate which set of Rf values is most likely to apply to naproxen.



Naproxen

(i) 1. 18, 2. 25, 3. 24; (ii) 1. 40, 2. 25, 3. 14; (iii) 1. 33, 2. 45, 3. 14; (iv) 1. 20, 2. 28, 3. 10

(iii) Answer

## Modification of TLC adsorbant

### Treatment of silica gel with KOH

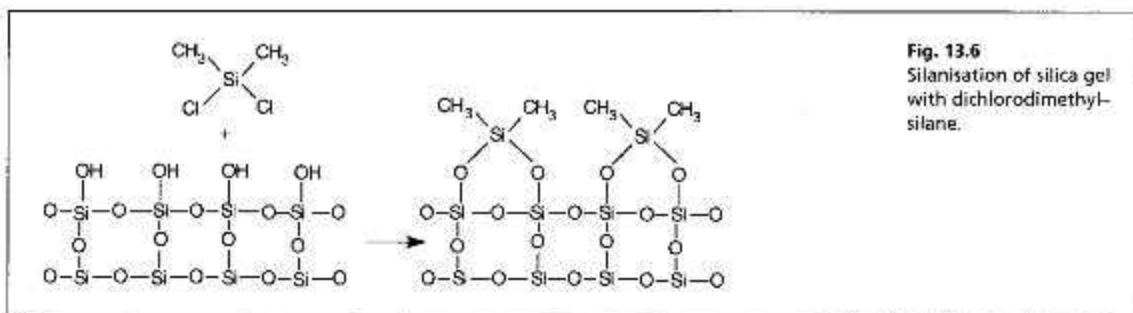
For analysis of basic compounds, silica gel which has been sprayed with a solution of KOH in methanol, may be used. Treating the plate with base ensures that basic compounds chromatograph as their free bases rather than as their salts. The salts of the amines have very low mobility in organic solvent-based mobile phases since basic compounds tend to interact strongly with silanol groups on the surface of the silica; the presence of KOH in the stationary phase suppresses this interaction. The mobile phases used in these type of systems also typically contain a basic component. Examples of the mobile phases used for the analysis of basic drugs on KOH impregnated silica gel include:

- Methanol/strong ammonia solution (100:1.5)
- Cyclohexane/toluene/diethylamine (75:15:10)
- Chloroform/methanol (90:10).

System 2 is quite non-polar and useful for discriminating between highly lipophilic bases, which include many of the antihistamines and narcotics, and sympathomimetic bases, which are often quite polar and move very little in mobile phase 2. The use of selective solvent systems is often combined with use of location agents that are selective for nitrogenous drugs.

## Silanised silica gel

The surface of the silica gel can be rendered non-polar by reaction with dichlorodimethylsilane as shown in Figure 13.6. A wide range of silanising reagents can be used in this type of reaction including octadecylsilanes, which produce ODS silica gel plates analogous to ODS HPLC columns. The BP uses silanised silica gel TLC plates in identity tests for penicillins. For example, a 0.25% w/v solution of ampicillin test material is applied to a silanised silica gel plate along with an ampicillin reference standard (0.25% w/v), Solution 2, and a mixture containing reference standards for ampicillin and amoxycillin trihydrate (0.25% w/v), Solution 3. The plate is developed with a mobile phase consisting of a solution of ammonium acetate adjusted to pH 5.0 with acetic acid and acetone (90:10). After development, the plate is stained with iodine vapour; the identity test specifies that the test substance should give a single spot with the same  $R_f$  as that seen for Solution 2 and that Solution 3 should show two clearly separated spots.



**Fig. 13.6**  
Silanisation of silica gel  
with dichlorodimethyl-  
silane.

This type of test could be carried out equally well with commercially produced ODS plates. Silica gel plates can be simply modified with reaction with organosilane reagents, the availability of a wide range of reactive organosilanes means that there is potential for producing a wide range of coated TLC plates for specific purposes.

## Keiselguhr as an inert support

Keiselguhr in itself does not have strong absorptive properties but it can be coated with a liquid or waxy stationary phase. The keiselguhr coated with liquid paraffin is used in a pharmacopoeial test for triglycerides and fatty acids in fixed oils. The keiselguhr plate is impregnated with a solution containing liquid paraffin in petroleum ether. This renders the surface hydrophobic. The samples of fixed oil being examined are applied to the plate and the plate is developed with acetic acid as the mobile phase. Acetic acid is a very polar solvent and thus the liquid paraffin stationary phase does not dissolve in it appreciably. Furthermore, the triglycerides in the fixed oil are only weakly polar and will partition usefully between the liquid paraffin stationary phase and the acetic acid mobile phase. The longer the chain length of the fatty acids in the triglyceride the lower the  $R_f$  of the triglyceride. The plate is visualised by staining with iodine and then permanently staining the iodine spots with starch solution. The BP shows the typical chromatograms that would be obtained from a number of fixed oils which are composed of mixtures of triglycerides in different proportions. The triglyceride composition of a particular fixed oil does not vary greatly and is very characteristic. A similar test to the one described above is carried out for the fatty acids composing the oil following hydrolysis of the triglycerides.



Other agents used to impregnate keiselguhr include: formamide and propan-1,2-diol. In the case of these impregnating agents, the mobile phases used to develop the treated plates have to be of low polarity to avoid washing the agent off the plate.

## Detection of compounds on TLC plates following development

A wide range of methods can be used to detect compounds on a TLC plate following its development with a mobile phase.

### Ultraviolet light

In order to observe the absorption of UV light by an analyte, silica gel which has been impregnated with a fluorescent material is used to prepare the TLC plate. Light with a wavelength of 254 nm is used to illuminate the plate and if the analyte absorbs UV light it can be seen as a black spot on a yellow background where it quenches the fluorescence of the background. This method of visualisation is used in many pharmacopoeial tests since most drugs possess chromophores. If a compound is naturally fluorescent, longer wavelength light at 365 nm may be used to visualise the plate. For example, the pharmacopoeial test for anthraquinones in aloes observes the fluorescence of these compounds under UV light at 365 nm.

### Location reagents

There is a huge number of location reagents available and these reagents range from those which are fairly specific for a particular type of analyte to those which will detect many different compounds.

#### *Iodine vapour*

The plate is put into a tank containing iodine crystals. This treatment will produce brown spots with many organic compounds; the staining is reversible, so that if it is necessary to recover the compound once it has been located, the iodine may be allowed to evaporate by exposing the plate to air and then the marked spot containing the compound of interest may be scraped off the plate. If a permanent record of the plate is required it has to be covered to prevent the iodine evaporating or the iodine spots may be sprayed with starch solution in order to stain them permanently. Iodine is used as a location agent in pharmacopoeial TLC tests of fixed oils and of cetrimide.

#### *Potassium permanganate*

Potassium permanganate provides a method for the detection of sugars and sugar-like molecules, and drugs with aliphatic double bonds. It is used in TLC identity checks for the antibacterial agents clindamycin and lincomycin and in a check for related substances in spectinomycin.

#### *Ninhydrin solution*

This reagent gives pink spots with primary amines and yellow spots with tertiary amines. It is used in pharmacopoeial identity tests for some of the aminoglycoside antibiotics such as gentamycin, in a limit test for aminobutanol in ethambutol and can be used as a general screen for nitrogen-containing drugs in conjunction with Dragendorff reagent. Dragendorff reagent will produce orange spots with tertiary

amines and may be used to overspray plates which have been sprayed in the first instance with ninhydrin.

#### *Alkaline tetrazolium blue*

This reagent is quite specific for corticosteroids producing blue spots on a white background. The tetrazolium spray is used in a test for related foreign steroids in flucorolone acetoneide.

#### *Ethanol/sulphuric acid 20%*

This reagent is used to produce fluorescent spots from corticosteroids such as dexamethasone or prednisolone by spraying the plate, heating to 120°C and then observing the plate under UV light at 365 nm.

## Applications of TLC analysis

### Qualitative identity tests

TLC is often used by BP monographs as part of a number of identity tests performed on pure substances. For extra confirmation of identity, more than one solvent system may be used and also different types of spray reagent may be used. Some examples of identity checks based on TLC have been mentioned earlier. Table 13.4 lists a few of the compounds which have their identity checked by TLC and a variety of location reagents and mobile phases are used to illustrate the fact that there is much less uniformity about TLC methodology than there is in the case of HPLC or GLC methodology.

**Table 13.4** Some examples of identity tests based on TLC described in pharmacopoeial monographs

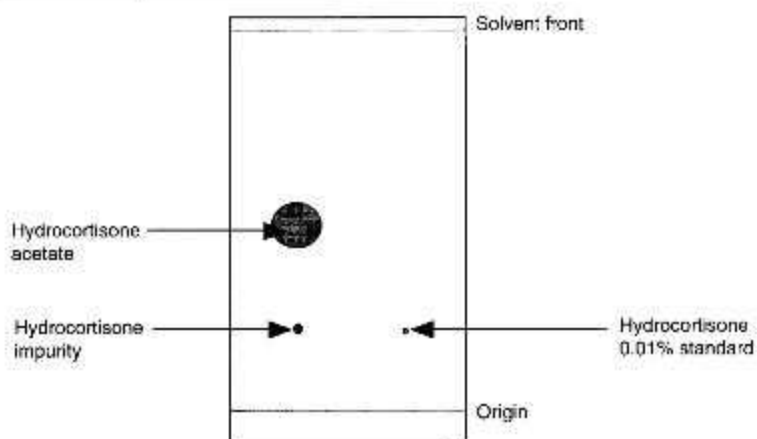
Substance examined	Stationary phase	Mobile phase	Visualisation reagent	Comments
Framycetin sulphate	Silica gel + carbomer binder	10% w/v $\text{KH}_2\text{PO}_4$	Naphthalene diol/ $\text{H}_2\text{SO}_4$	R <sub>f</sub> and colour of the sample are compared with a pure standard. The resolution of the analyte from streptomycin is checked
Methyl prednisolone	Silica gel GF <sub>254</sub>	Ether/toluene/butan-1-ol saturated with water (85:10:5)	UV light 254 nm then ethanolic sulphuric acid (20%) + heat to 120°C	R <sub>f</sub> and colours of the sample and standard are compared. Also R <sub>f</sub> of an oxidation product is used as an additional check
Aprotinin	Silica gel	Acetate buffer	Ninhydrin spray	R <sub>f</sub> and colour of the analyte spot is compared with standard
Levamisole	Silica gel H with fluorescent indicator	Toluene/acetone/13.5 M ammonia (60:40:1)	UV light 254 nm	R <sub>f</sub> and size of the spot obtained is matched to that of a standard.
Pentagastrin	Silica gel G	Analyte is examined by TLC in three different mobile phases	4-dimethyl-aminobenzaldehyde in methanol/HCl	The R <sub>f</sub> of the analyte in three different mobile phases is determined and the colour of its spot is matched to that of the standard

## Limit tests

### Where the structure of the impurity is known

TLC is used to perform limit tests for impurities in many pharmacopoeial monographs. A TLC limit test is based on comparison between a concentrated solution of an analyte and a dilute solution of an impurity. The intensities of the spots due to any impurities in the analyte are compared with the intensity of a spot or spots due to standards spotted separately onto the same plate. For the purposes of the examples illustrated as follows, intensity and size are regarded as being interchangeable, which they are to a large extent. For instance a limit test might be conducted for hydrocortisone in hydrocortisone acetate as follows: 5  $\mu\text{l}$  of 1% w/v solution of hydrocortisone acetate are spotted onto the origin of a TLC plate; at another position on the plate, 5  $\mu\text{l}$  of a 0.01% w/v of hydrocortisone are spotted on. The TLC plate is developed in the solvent described in Self-test 13.1 and might appear as shown in Figure 13.7 when viewed under UV light. In the example shown, a small amount of hydrocortisone impurity can be seen running below the large spot due to hydrocortisone acetate, which is the main component in the sample. In line with the position where the hydrocortisone standard was spotted onto the plate, there is a very faint spot. In this case the spot for the hydrocortisone impurity in the sample can be seen to be more intense (larger) than the spot due to the 0.01% w/v hydrocortisone standard and thus the sample has failed the limit test. This test is a 1% limit test since  $0.01/1 \times 100 = 1\%$ .

Fig. 13.7  
TLC limit test for  
hydrocortisone in  
hydrocortisone acetate.



### Self-test 13.4

A limit test is conducted for hydrocortisone in hydrocortisone sodium phosphate. 2  $\mu\text{l}$  of a 1% w/v solution of hydrocortisone sodium phosphate is compared with 2  $\mu\text{l}$  of a solution containing 0.02% w/v hydrocortisone standard using the solvent system given in Self-test 13.1.

- What is the percentage limit for hydrocortisone in hydrocortisone sodium phosphate set by this test?
- Would the spot for the impurity appear above or below the substance being examined? (see Self-test 13.1).

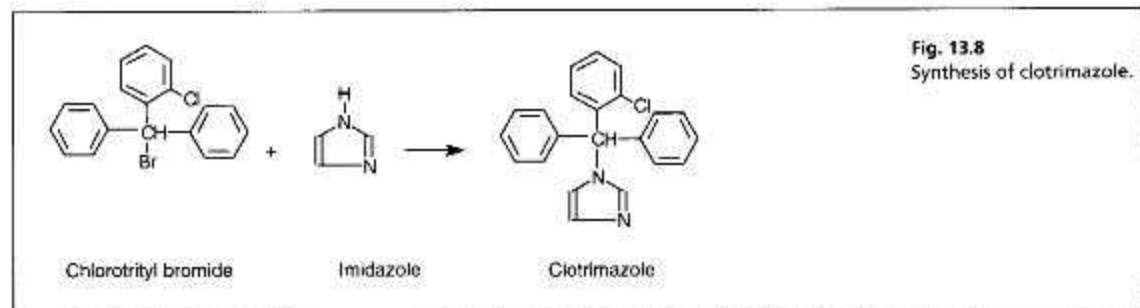
Answers: (i) 2%; (ii) Above

Table 13.5 shows some BP limit tests for known impurities used in pharmacopoeial monographs.

**Table 13.5** BP limit tests for known impurities in pharmaceutical raw materials

Test substance	Impurity	Limit set (%)
Clotrimazole	Chlorotritanol	0.2
Clotrimazole	Imidazole	0.2
Cyclizine	N-methylpiperazine	0.5
Dexpanthenol	3-aminopropanol	0.5
Ethinylloestradiol	Estrone	1.0
Loprazolam mesylate	N-methylpiperazine	0.25
Mefenamic acid	2,3-dimethyl aniline	0.01
Mexiletine hydrochloride	2,6-dimethyl phenol	0.2
Phenoxyethylpenicillin	Phenylacetic acid	0.5

As in the case of hydrocortisone acetate, where hydrocortisone might be expected to occur as a result of hydrolysis of the acetate ester, tests for the presence of known impurities are based on the known manufacturing sequence or on likely degradation pathways. For example, the tests carried out on clotrimazole are based on the last step in its manufacture as shown in Figure 13.8. Unreacted imidazole is an obvious impurity and chlorotritanol would be readily formed from unreacted chlorotrityl bromide by hydrolysis, which would occur when the clotrimazole is extracted from the reaction mixture.



**Fig. 13.8**  
Synthesis of clotrimazole.

Most of the examples given in Table 13.5 have similar obvious origins in the drug manufacturing process.

#### *Where the structure of the impurity is unknown*

A related type of TLC limit test is carried out where the identities of impurities are not completely certain. This type of test is used, for instance, on compounds of natural origin or partly natural origin which may contain a range of compounds related in structure to the test substance which are co-extracted with the raw starting material. For example, the range of synthetic steroids originate from triterpenoids extracted from plants, which are extensively modified by fermentation and chemical synthesis.

The assumption which is made in the type of test described following is that the related unknown substances will produce a similar intensity of spot to the test substance itself at equal concentrations. For example, a limit test is conducted for related (foreign) alkaloids in codeine, which is extracted from the opium poppy in which a range of alkaloids occur: thus, the exact identity of the impurities may not be known. To conduct the test, 10  $\mu$ l amounts of three solutions are applied separately to a TLC plate. The solutions contain: 4.0% w/v codeine (Solution 1),

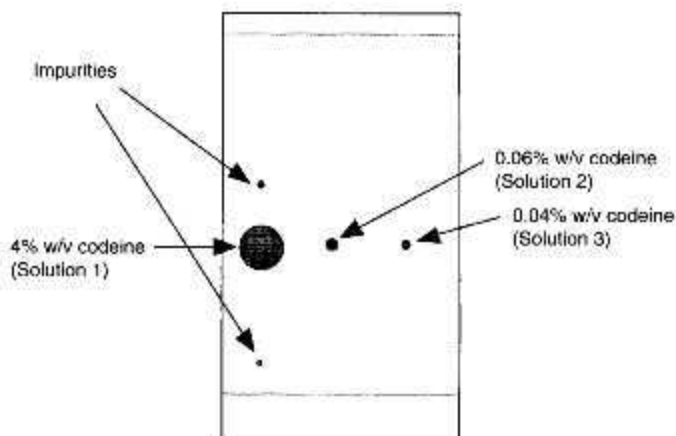
0.06% w/v codeine (Solution 2) and 0.04% w/v codeine (Solution 3). In the test, the dilute solutions of codeine are used as visual comparators for any impurities in the sample. The plate is developed in ethanol/cyclohexane/13.5 M ammonia (72:30:6), is dried and is then sprayed with iodobismuthate reagent, which is specific for nitrogenous drugs. After development and spraying the plate might look like the diagram shown in Figure 13.9. The conditions set by the limit test are that:

- (i) There should be no secondary spot in the chromatogram of Solution 1 which is more intense than the spot obtained with Solution 2.
- (ii) There should be no more than one secondary spot, with a  $R_f$  value higher than that of codeine, which is more intense than the spot obtained with Solution 3.

In this test, two limits are being set:  $0.06/4 \times 100 = 1.5\%$  and  $0.04/4 \times 100 = 1.0\%$ .

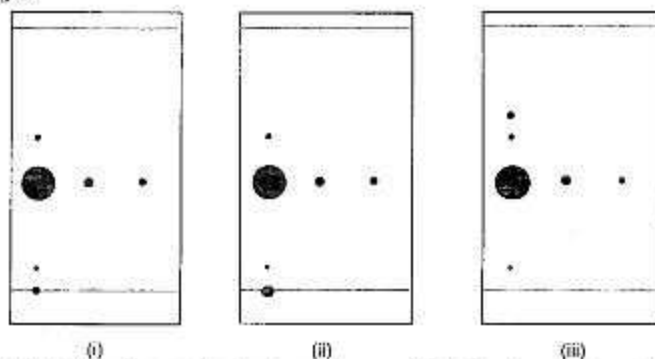
This type of test can be a little confusing at first since there are a number of permutations that can lead to the sample passing or failing of the test.

**Fig. 13.9**  
TLC limit test for  
impurities in codeine.



### Self-test 13.5

Three samples of codeine are analysed as described earlier, which indicate whether the TLC limit tests shown below pass or fail the samples. Solutions 1–3 appear in numerical order from left to right.



- (i) Passes since there is no spot in the chromatogram of Solution 1 more intense than the spot due to Solution 2.
- (ii) Fails since the spot at origin is more intense than the spot due to Solution 2.
- (iii) Passes since, although one spot above codeine is more intense than the spot due to Solution 3, there is still no spot more intense than the spot due to Solution 2.

## Tests in which known and unknown standards are used

Table 13.6 shows some of the other limit tests set in pharmacopoeial monographs ranging from a simple test for a known impurity to tests in which limits are set for more than one known impurity plus any unknown impurities which might be present.

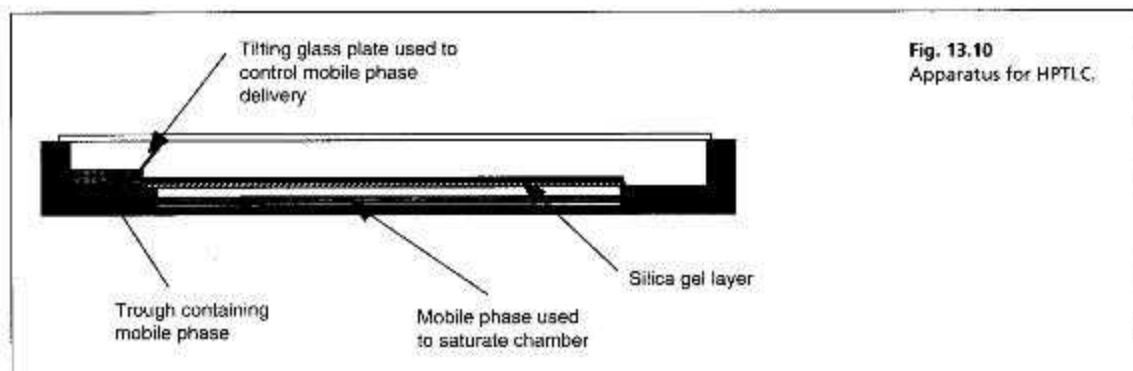
**Table 13.6** Some examples of pharmacopoeial limit tests

Analyte solution (Solution 1)	Limit test (Solution 2)	Limit test (Solution 3)	Limit test (Solution 4)
10 % w/v procaine.HCl	No secondary spot > 0.005% w/v <i>p</i> -amino benzoic acid	–	–
1% w/v triamcinolone acetonide	No secondary spot > 0.02% w/v triamcinolone acetonide	No other spots > 0.01% w/v triamcinolone acetonide	–
2% w/v promethazine.HCl	No secondary spot > 0.02% w/v isopromethazine.HCl	No other spots > 0.01% w/v promethazine.HCl	–
1% w/v chloramphenicol palmitate	No spot with the same $R_f$ > 0.02% w/v chloramphenicol palmitate isomer.	No spot with the same $R_f$ > 0.02% w/v chloramphenicol dipalmitate	No other spots > 0.005% w/v chloramphenicol palmitate

Perhaps the most detailed pharmacopoeial limit test of this nature is carried out on tetracycline where a 1% w/v solution is spotted onto the TLC plate with solutions of five structurally related tetracyclines ranging in concentration from 0.02–0.005% w/v.

## High performance TLC (HPTLC)

HPTLC is conducted on TLC plates which are coated with purified silica gel with a particle range of 2–10  $\mu\text{m}$  as opposed to 2–25  $\mu\text{m}$  for standard commercial TLC plates. The narrower particle size range means that a greater number of theoretical plates are available for separation and thus the spots on the TLC plate remain tighter. These type of plates may be run in a standard type of TLC tank but optimal performance is obtained from horizontal development of the plates using apparatus of the type shown in Figure 13.10.



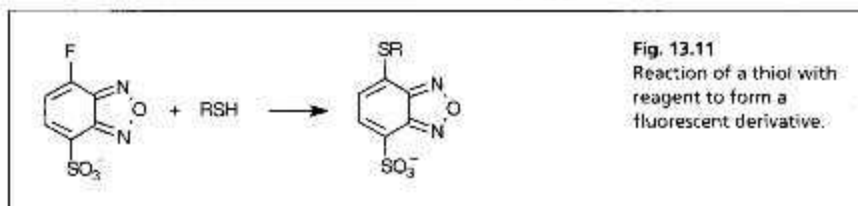
**Fig. 13.10**  
Apparatus for HPTLC.

The advantages of horizontal development are:

- (i) The mobile phase moves more quickly.
- (ii) The proximity of the plate's surface to a saturating solution of mobile phase means that there is little evaporation of solvent from the surface of the plate, which in the case of vertical development can change the composition of the mobile phase as it moves up the plate.
- (iii) In the vertical position, if the plate is not in a saturated atmosphere, solvent at the edge of the plate tends to evaporate drawing solvent from the centre of the plate and causing the solvent at the edge of the plate to migrate more quickly. This does not occur when horizontal development is used.

## Applications of HPTLC

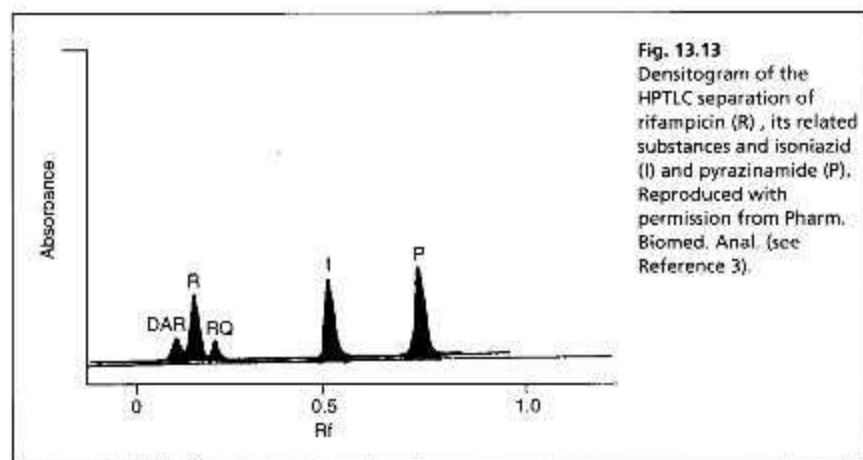
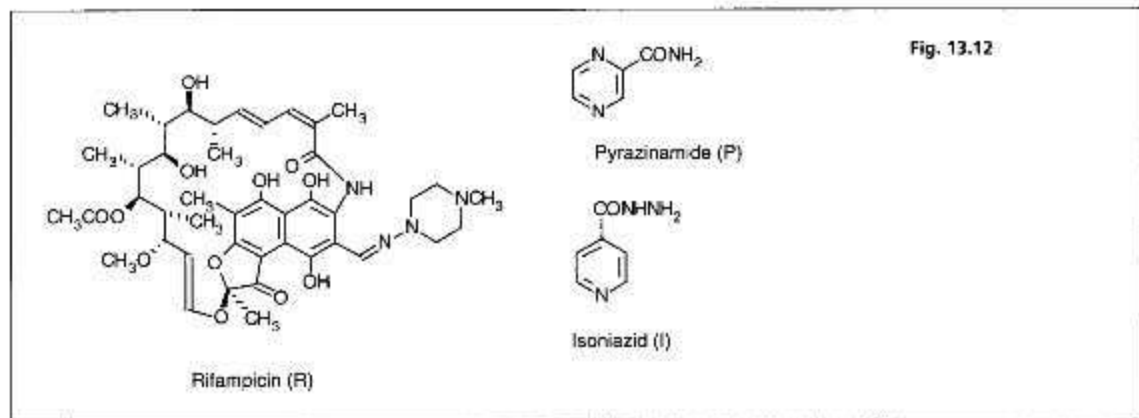
It is possible to use TLC as a quantitative method by using a densitometer to read spot intensity. Quantitative TLC is best carried out using high-performance systems. Densitometers can be used to quantify components in a sample on the basis of fluorescence or absorption of UV light. As discussed above there are a number of advantages in using TLC and a major advantage is the ability to run batches of samples which give it an advantage over HPLC. HPTLC with fluorescence densitometry has been applied to the analysis of pharmacologically active thiols including the ACE inhibitor captopril.<sup>3</sup> Compounds such as captopril do not have a strong chromophore and thus require derivatisation to render them detectable and this would be true whether HPLC or HPTLC were being used. In this example, the thiols were reacted with a thiol-specific reagent which produced fluorescent derivatives (Fig. 13.11) and were then analysed by TLC. Limits of detection for these compounds by this method were in the low picogram range.



**Fig. 13.11**  
Reaction of a thiol with reagent to form a fluorescent derivative.

An HPTLC assay for rifampicin (R), isoniazid (I) and pyrazinamide (P) (Fig. 13.12) in a single dosage form was reported.<sup>4</sup> Pharmacopoeial methods only allow for the determination of each analyte in separate dosage forms. The HPTLC method was also able to resolve rifampicin from two of its named impurities. The analytes were quantified by densitometry by measuring absorbance at two different wavelengths. The precisions reported for quantification of the analytes were  $R \pm 1.73$ ,  $I \pm 1.58$  and  $P \pm 1.07$ , which compared quite favourably with an HPLC method for the same dosage form. Figure 13.13 shows a densitogram for R, its related substances, desacetyl rifampicin (DAR) and rifampicin quinone (RQ) and I and P. Thus HPTLC can be used for the quantitative analysis of mixtures which include large and complex molecules such as rifampicin.

It would be possible to run many current pharmaceutical limit tests with a much higher degree of accuracy and precision if HPTLC methods were used.



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